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Report Title

Final Progress Report for W911NF-05-1-0432, Hibernation Strategies to Improve Recovery from Hemorrhagic Shock

ABSTRACT

The ultimate goal of this project is to protect the warfighter from pathology that occurs as a result of significant blood loss. The overall strategy is to develop an effective fast-acting hemorrhagic shock protection fluid based on the molecular mechanisms used by hibernating mammals to survive reduced blood flow and avoid the consequences of ischemia and reperfusion injury.

The primary deliverable derived from this Phase 1 project is the ability to protect a non-hibernating mammal against injury from hemorrhagic shock. We have already shown in preliminary experiments that ischemic rat livers are protected from damage in vivo by administration of a preconditioning solution based on a molecular profile seen in hibernators. Optimization of a hemorrhagic shock protection fluid in non-hibernating rats, and assaying for their ability to protect against hemorrhagic shock, will serve as a prelude to Phase 2 of the Surviving Blood Loss Program.

The ultimate goal of our work is to protect the warfighter from pathology that occurs as a result of significant blood loss. This effort will concentrate on the preconditioning protection of two organs that are critical for successful recovery from hemorrhagic shock, the heart and brain.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Number of Papers published in peer-reviewed journals: 0.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Klein, A., Drewes, L.R., and Andrews, M.T. (2006) Hibernation Strategies to Improve Recovery from Hemorrhagic Shock. APS Conference on Comparative Physiology, Virginia Beach, VA, October 8-11, 2006, The Physiologist 49, C1-47, Abstract #27.1.

Number of Presentations: 1.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

Number of Manuscripts:

Number of Inventions:

Graduate Students

NAME	PERCENT_SUPPORTED	
Amanda Klein	0.50	No
FTE Equivalent:	**0.50**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Matthew T. Andrews	0.25	No
Lester R. Drewes	0.10	No
FTE Equivalent:	**0.35**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	
Scott Wendroth	0.25	No
FTE Equivalent:	**0.25**	
Total Number:	**1**	

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

ARO Final Report
W911NF-05-1-0432
January 2007

Matthew T. Andrews, P.I.

Lester R. Drewes, Co-P.I.

Accomplishments Since Last Report

- Acute Blood Loss Experiments using D-BHB
  - Physiological monitoring completed
    - MABP, T_b, and Heart Rate (BPM)
  - New data from animals completed

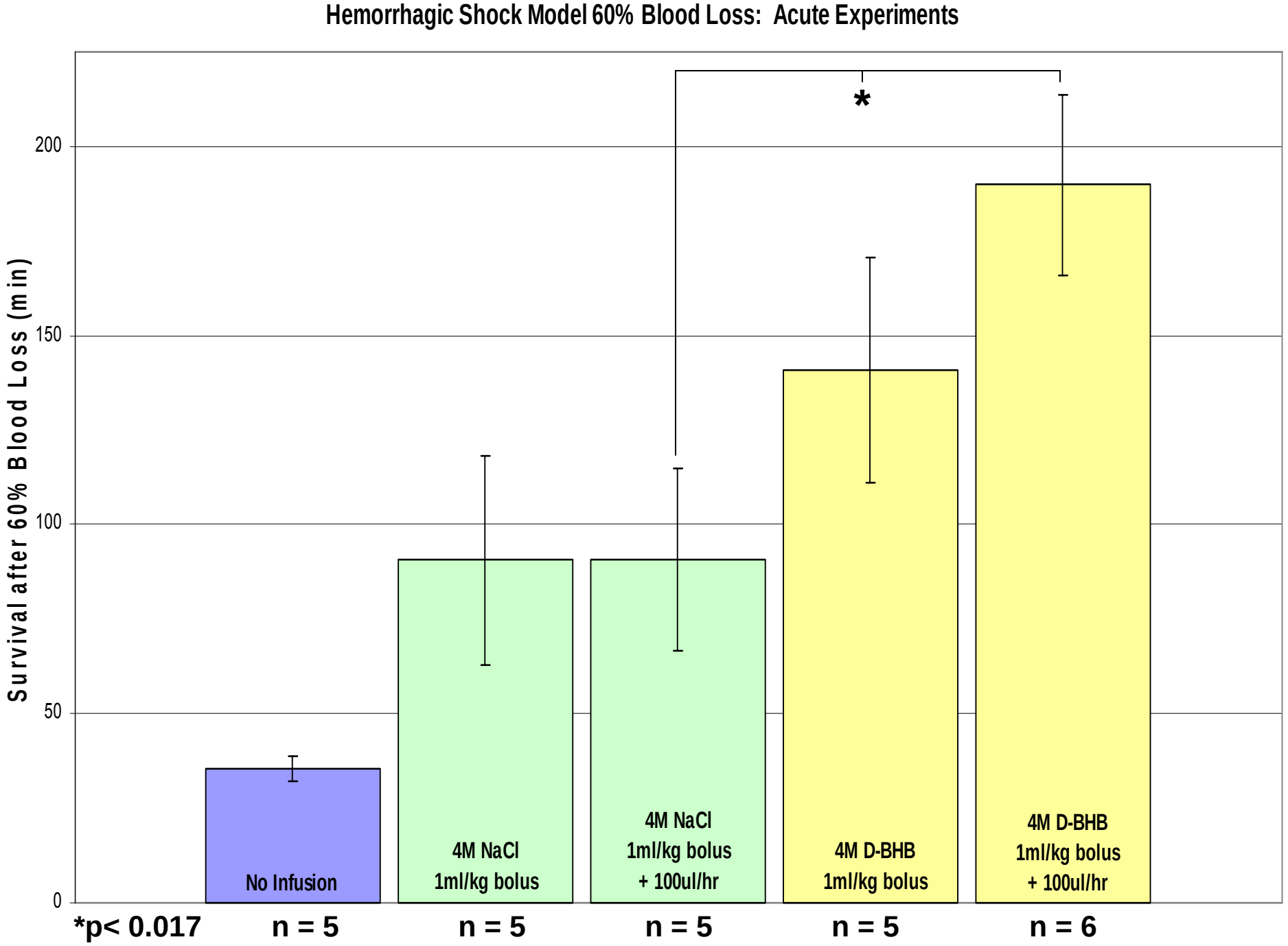
- Survival Following Blood Return using D-BHB
  - Physiological monitoring completed
  - New data from animals completed

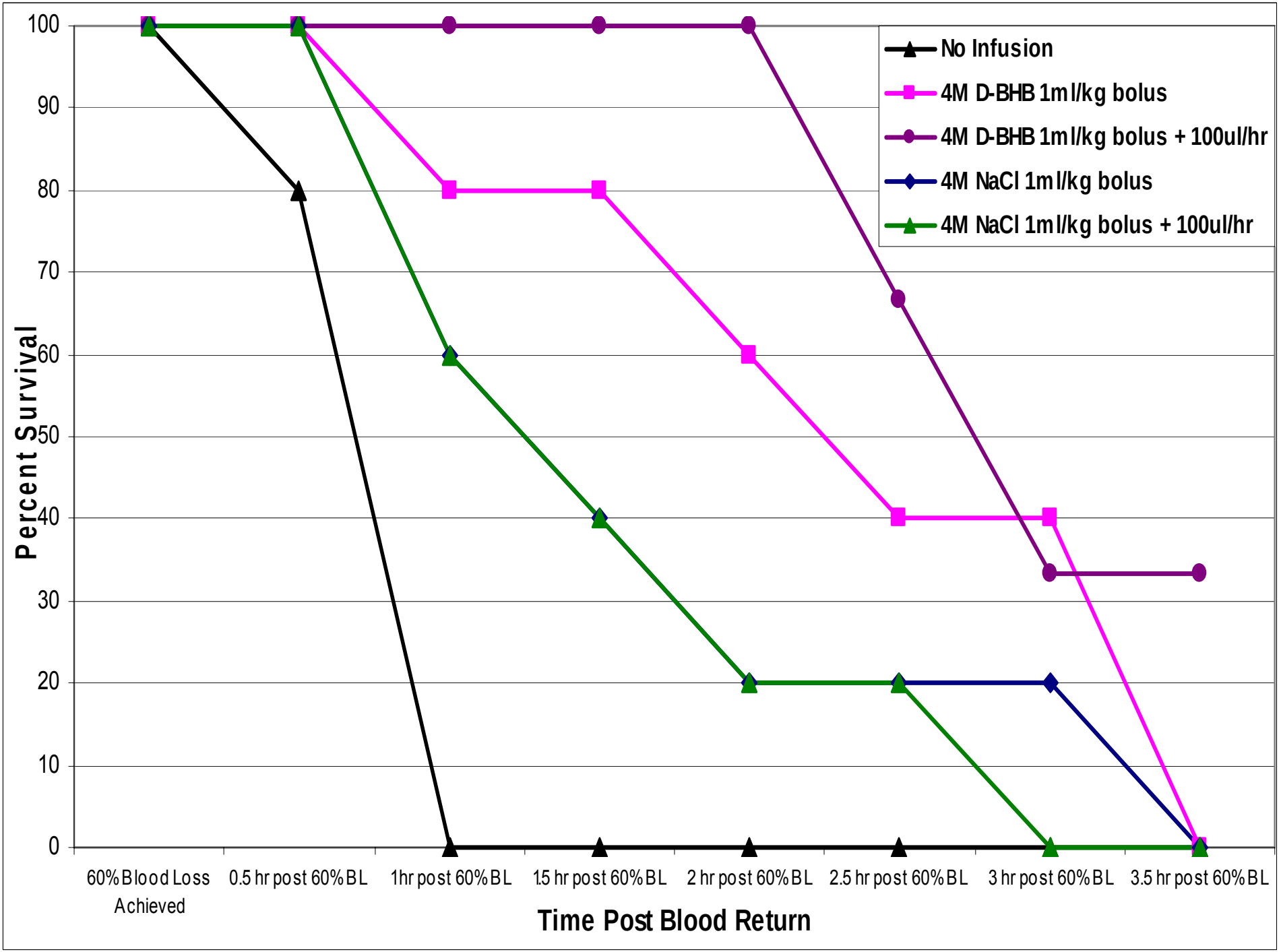
Acute Experiments

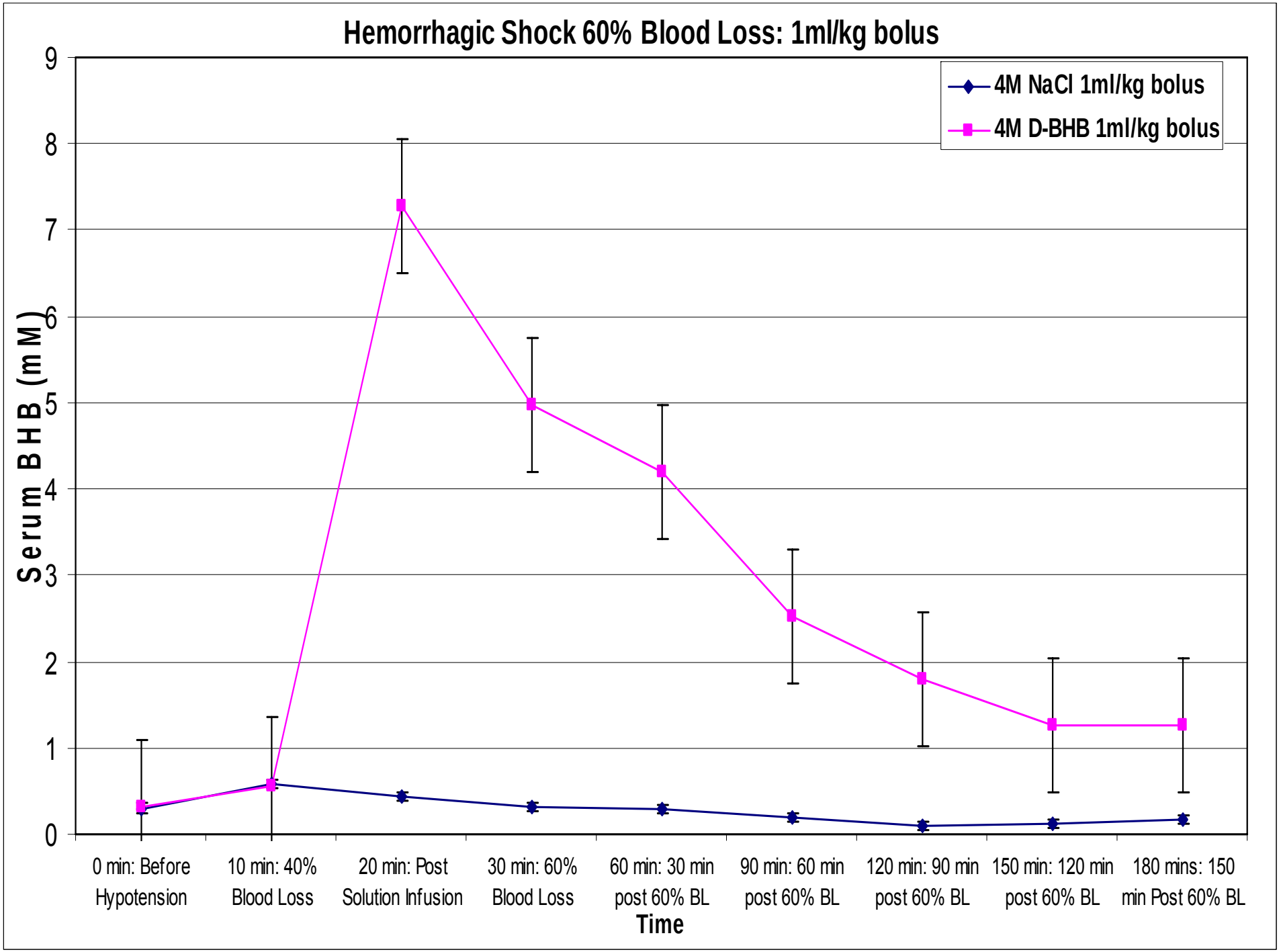
- Revised data on rats subjected to 60% blood loss
- Body temperatures allowed to cool to 27-29^{o}C
- Therapies:
  - Control (no infusion)
  - 4M NaCl 1ml/kg bolus
  - 4M NaCl 1ml/kg bolus + 100 µl/hr infusion
  - 4M D-BHB 1ml/kg bolus
  - 4M D-BHB 1ml/kg bolus + 100 µl/hr infusion

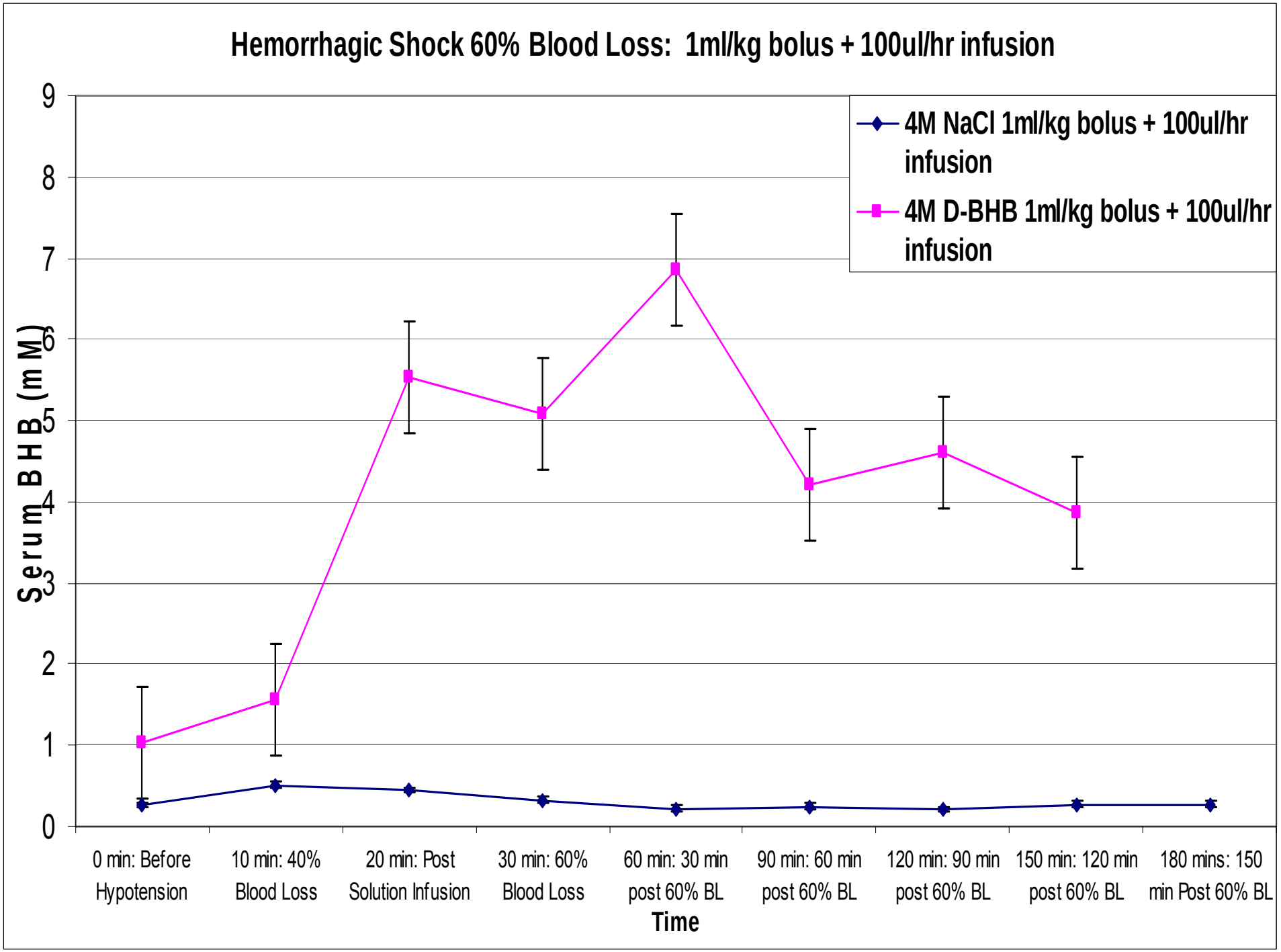
Acute Experiments

- 60% blood loss for 3 hours for a typical 300 g rat:
  - 10.8 ml blood removal
  - 600 µl solution replaced (~3.3% of original blood volume)


Hemorrhagic Shock Model 60% Blood Loss: Acute Experiments
Survival after 60% Blood Loss (min)
0
50
100
150
200
*
No Infusion
4M NaCl
1ml/kg bolus
4M NaCl
1ml/kg bolus
+ 100ul/hr
4M D-BHB
1ml/kg bolus
4M D-BHB
1ml/kg bolus
+ 100ul/hr
*p< 0.017
n = 5
n = 5
n = 5
n = 5
n = 6

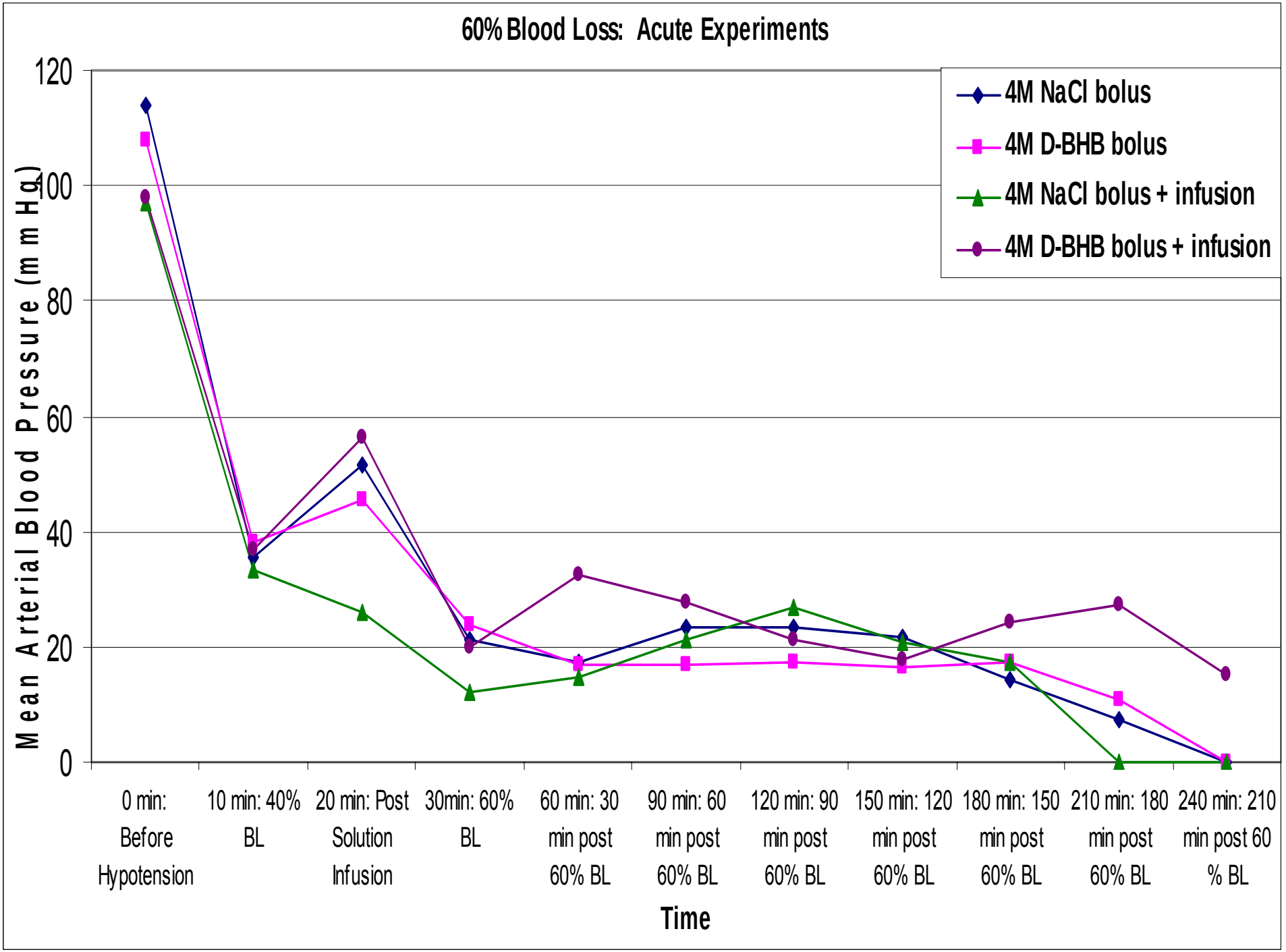

100
90
80
70
60
50
40
30
20
10
0
Percent Survival
No Infusion
4M D-BHB 1ml/kg bolus
4M D-BHB 1ml/kg bolus + 100ul/hr
4M NaCl 1ml/kg bolus
4M NaCl 1ml/kg bolus + 100ul/hr
60% Blood Loss Achieved
0.5 hr post 60% BL
1hr post 60% BL
1.5 hr post 60% BL
2 hr post 60% BL
2.5 hr post 60% BL
3 hr post 60% BL
3.5 hr post 60% BL
Time Post Blood Return

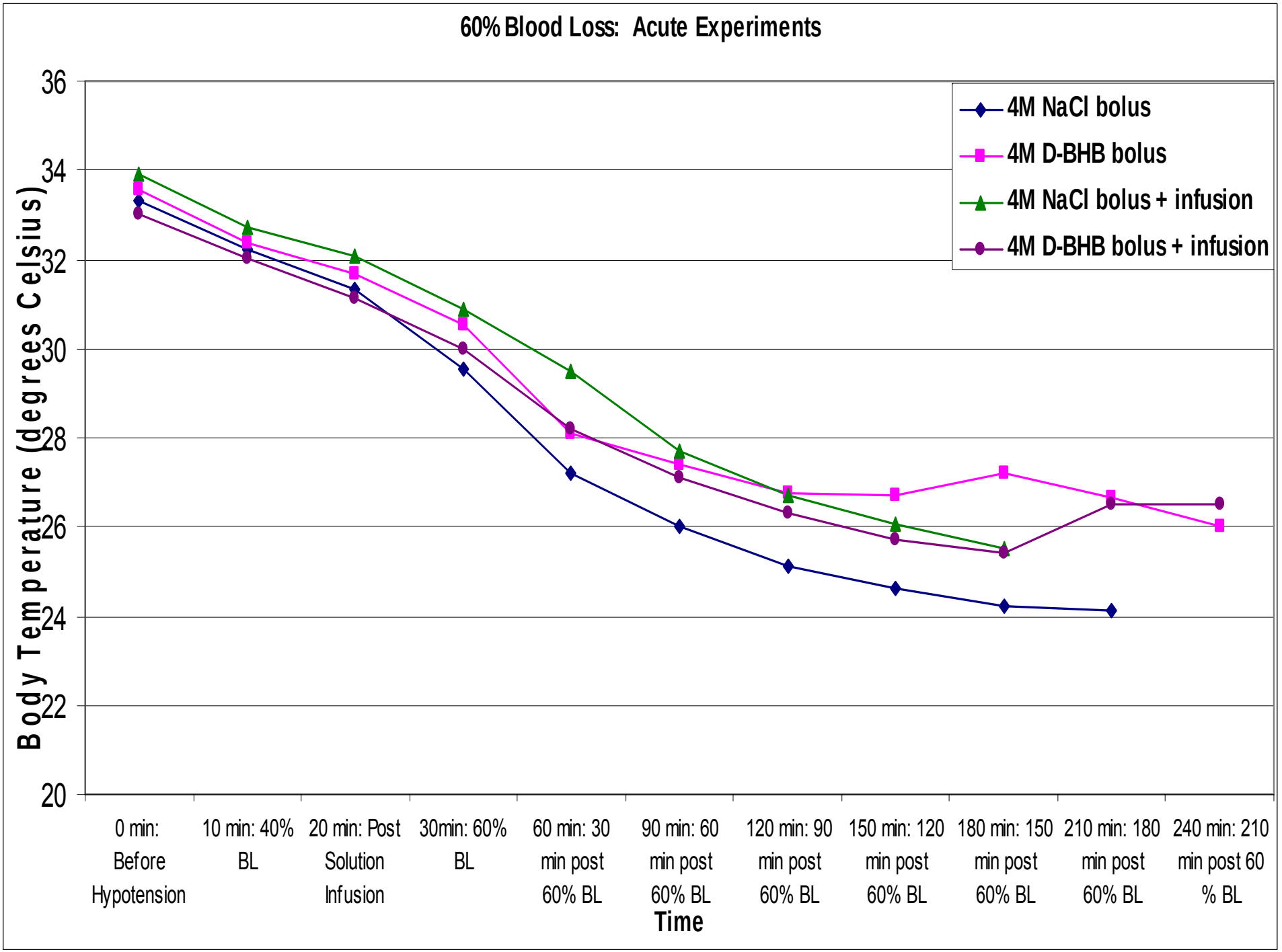

Hemorrhagic Shock 60% Blood Loss: 1ml/kg bolus
4M NaCl 1ml/kg bolus
4M D-BHB 1ml/kg bolus
Serum BHB (mM)
0
1
2
3
4
5
6
7
8
9
0 min: Before Hypotension
10 min: 40% Blood Loss
20 min: Post Solution Infusion
30 min: 60% Blood Loss
60 min: 30 min post 60% BL
90 min: 60 min post 60% BL
120 min: 90 min post 60% BL
150 min: 120 min post 60% BL
180 mins: 150 min Post 60% BL
Time

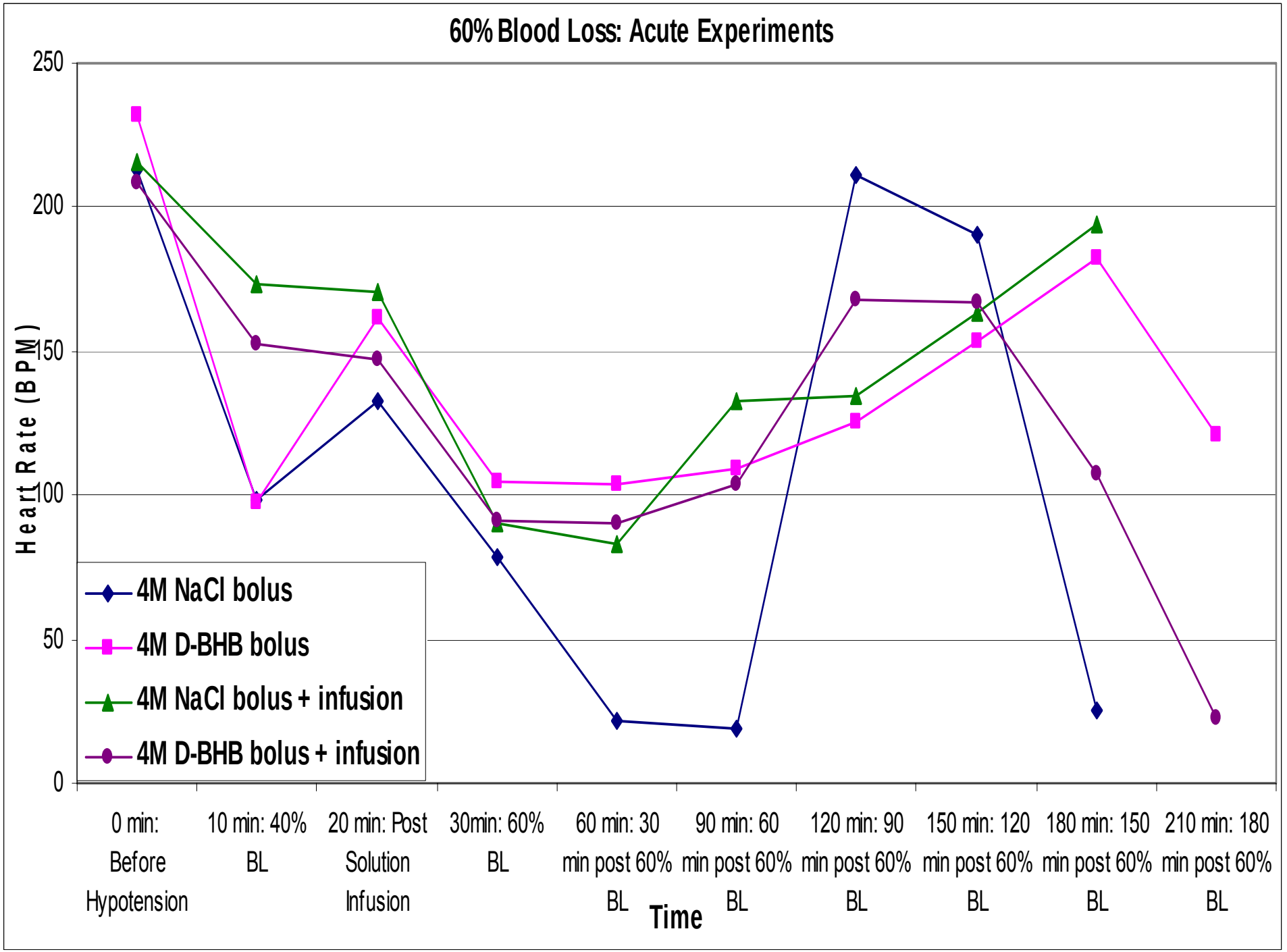

Hemorrhagic Shock 60% Blood Loss: 1ml/kg bolus + 100ul/hr infusion
4M NaCl 1ml/kg bolus + 100ul/hr infusion
4M D-BHB 1ml/kg bolus + 100ul/hr infusion
Serum BHB (mM)
0
1
2
3
4
5
6
7
8
9
0 min: Before Hypotension
10 min: 40% Blood Loss
20 min: Post Solution Infusion
30 min: 60% Blood Loss
60 min: 30 min post 60% BL
90 min: 60 min post 60% BL
120 min: 90 min post 60% BL
150 min: 120 min post 60% BL
180 mins: 150 min Post 60% BL
Time

Physiological Monitoring: Acute Experiments

- What physiological parameters are improved upon infusion with D-BHB?
  - Mean Arterial Blood Pressure (MABP)
  - Body Temperature (T_b)
  - Heart Rate (BPM)
- Are these different from NaCl control?


60% Blood Loss: Acute Experiments
Mean Arterial Blood Pressure (m m Hg)
120
100
80
60
40
20
0
4M NaCl bolus
4M D-BHB bolus
4M NaCl bolus + infusion
4M D-BHB bolus + infusion
0 min: Before Hypotension
10 min: 40% BL
20 min: Post Solution Infusion
30min: 60% BL
60 min: 30 min post 60% BL
90 min: 60 min post 60% BL
120 min: 90 min post 60% BL
150 min: 120 min post 60% BL
180 min: 150 min post 60% BL
210 min: 180 min post 60% BL
240 min: 210 min post 60 % BL
Time


60% Blood Loss: Acute Experiments
Body Temperature (degrees Celsius)
36
34
32
30
28
26
24
22
20
4M NaCl bolus
4M D-BHB bolus
4M NaCl bolus + infusion
4M D-BHB bolus + infusion
0 min: Before Hypotension
10 min: 40% BL
20 min: Post Solution Infusion
30min: 60% BL
60 min: 30 min post 60% BL
90 min: 60 min post 60% BL
120 min: 90 min post 60% BL
150 min: 120 min post 60% BL
180 min: 150 min post 60% BL
210 min: 180 min post 60% BL
240 min: 210 min post 60 % BL
Time


60% Blood Loss: Acute Experiments
Heart Rate (BPM)
250
200
150
100
50
0
4M NaCl bolus
4M D-BHB bolus
4M NaCl bolus + infusion
4M D-BHB bolus + infusion
0 min: Before Hypotension
10 min: 40% BL
20 min: Post Solution Infusion
30min: 60% BL
60 min: 30 min post 60% BL
90 min: 60 min post 60% BL
120 min: 90 min post 60% BL
150 min: 120 min post 60% BL
180 min: 150 min post 60% BL
210 min: 180 min post 60% BL
Time

Acute Experiment Conclusions

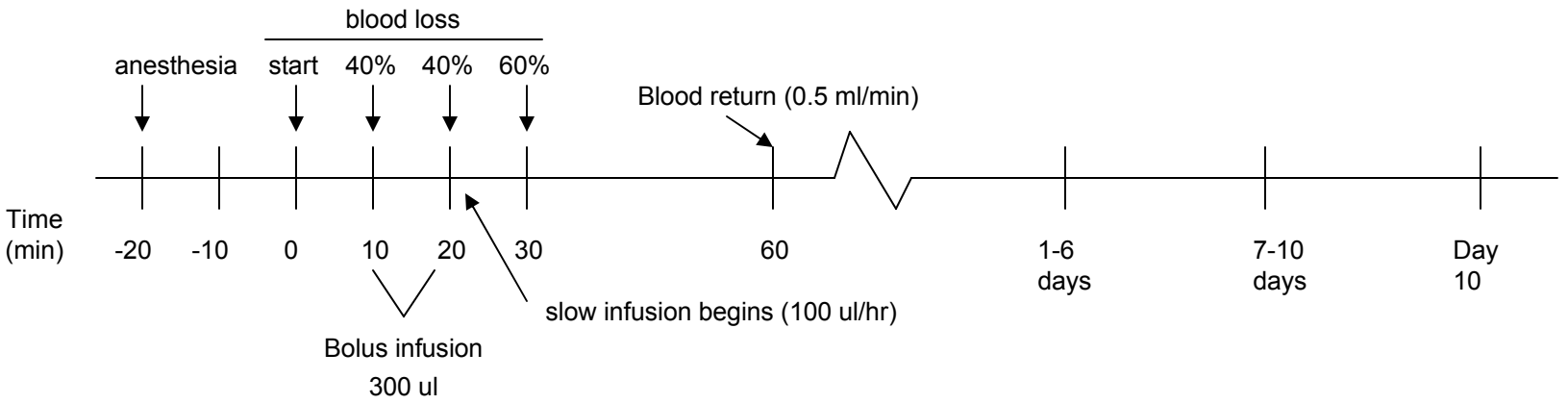
- Serum levels of BHB increase upon infusion
- Mean concentration of circulating BHB as high as 7 mM
- Maintaining elevated concentration of BHB prolongs survival of 60% blood loss to approx. 3hrs (mean = 189 min)
- Physiological parameters of MABP, T_b, and Heart Rate are generally the same with ketone and control solutions

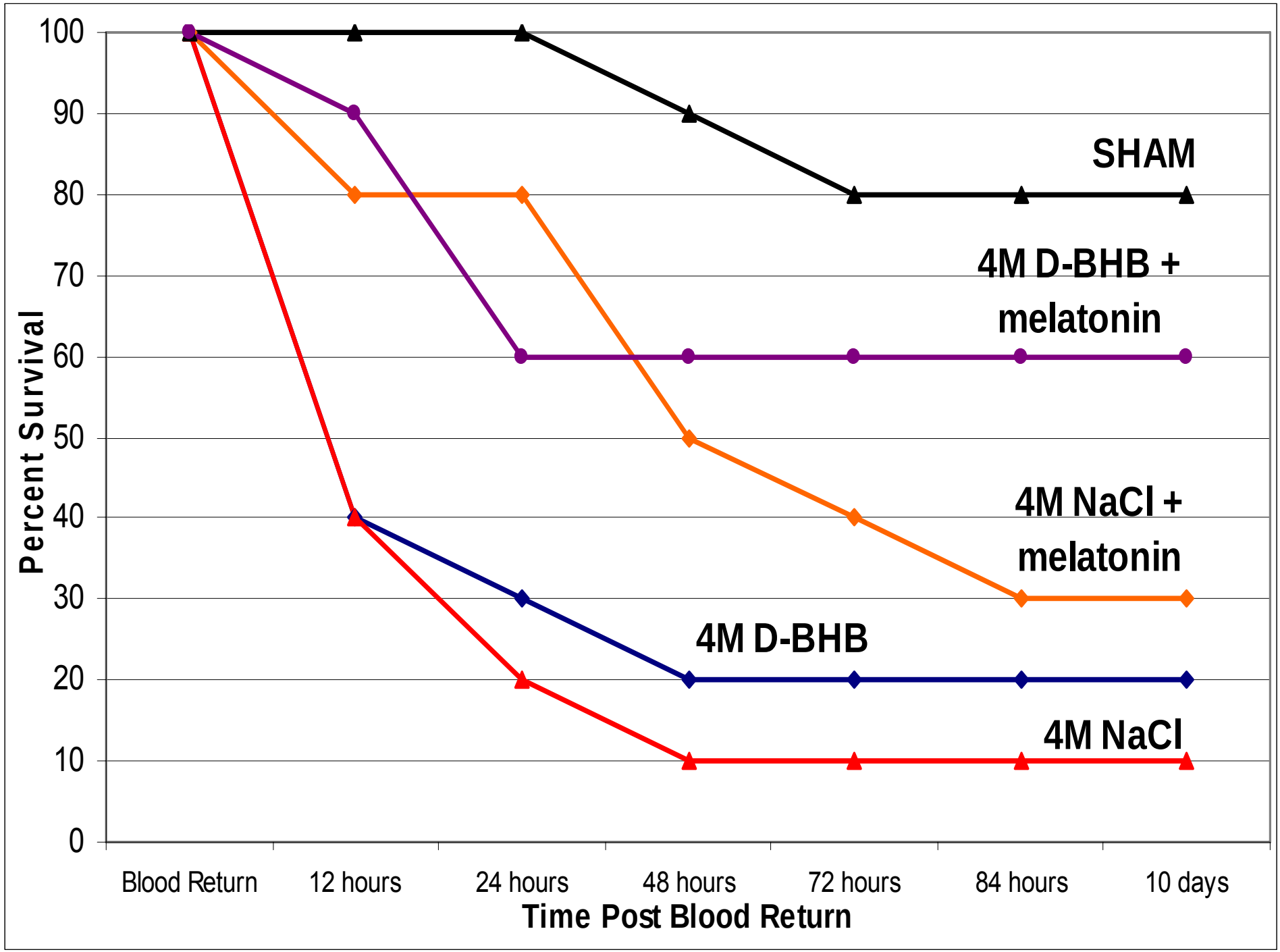
Survival Experiments

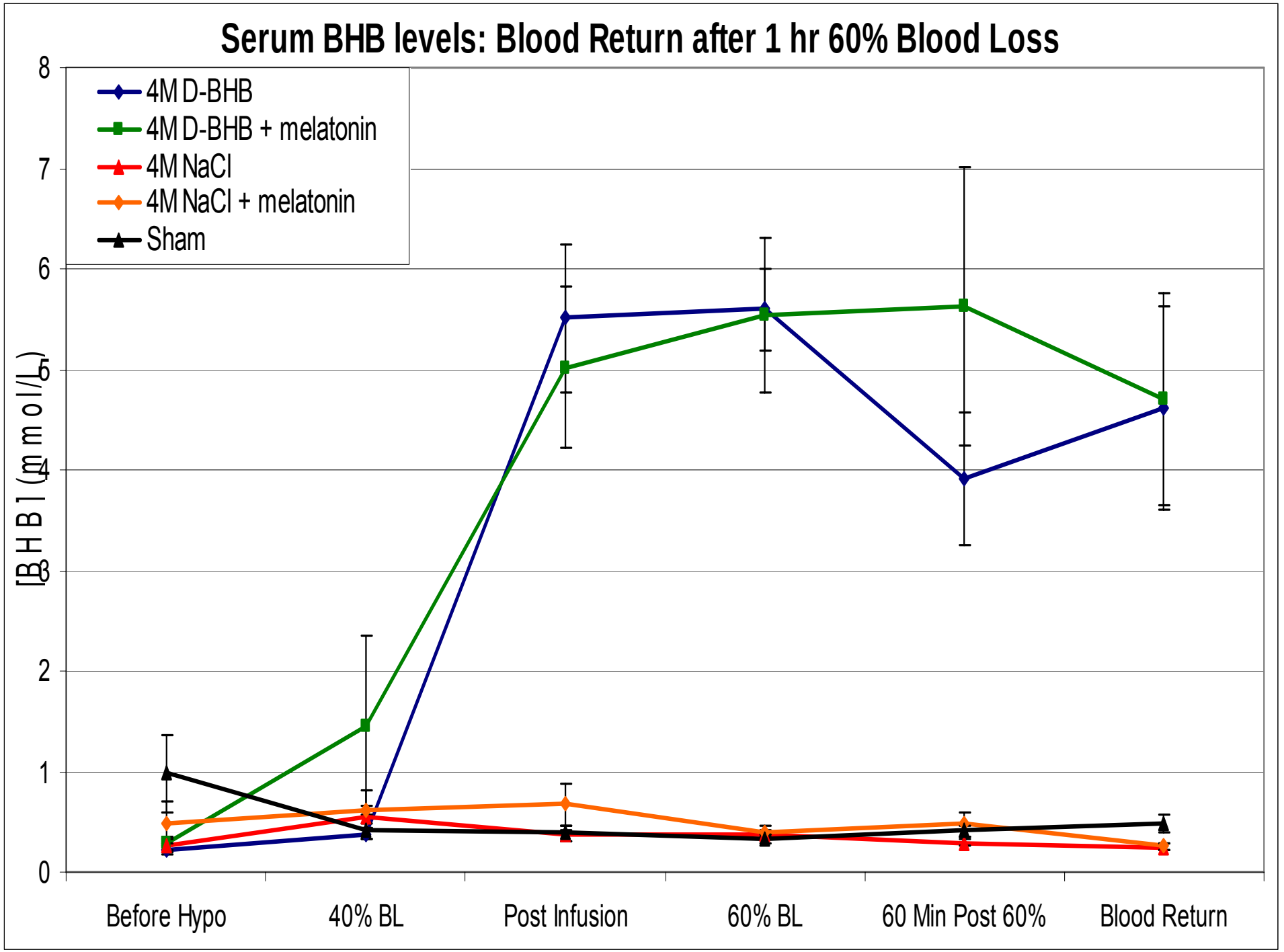
- Animals given shed blood after one hour post 60% blood loss achieved
- Blood return at 0.5 ml/min
- Temperature of shed blood is at the animal's body temperature ($27^{o}C$ – $29^{o}C$) when returned
- Animals allowed to recover
- SHAM animals: No blood loss, received anesthesia; and blood samples taken at equivalent time points

Blood Return Experiments

- Return shed blood after 1 hr at 60% BL
- Monitor rats quality of life post blood return
  - Day 1-6: Neurological Scoring
  - Day 7-10: Memory Testing
  - Day 10: Sacrifice → Brain Histology

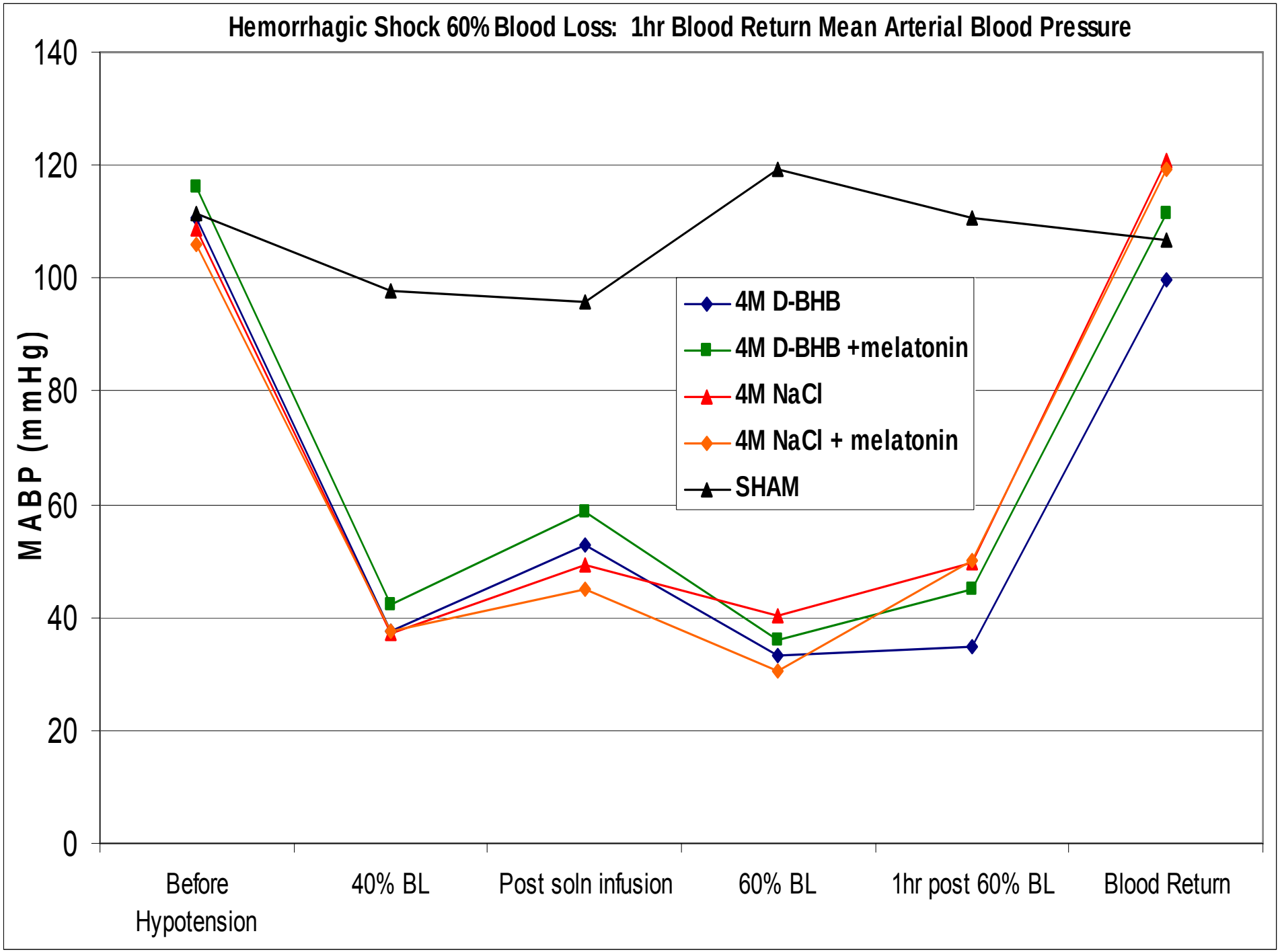


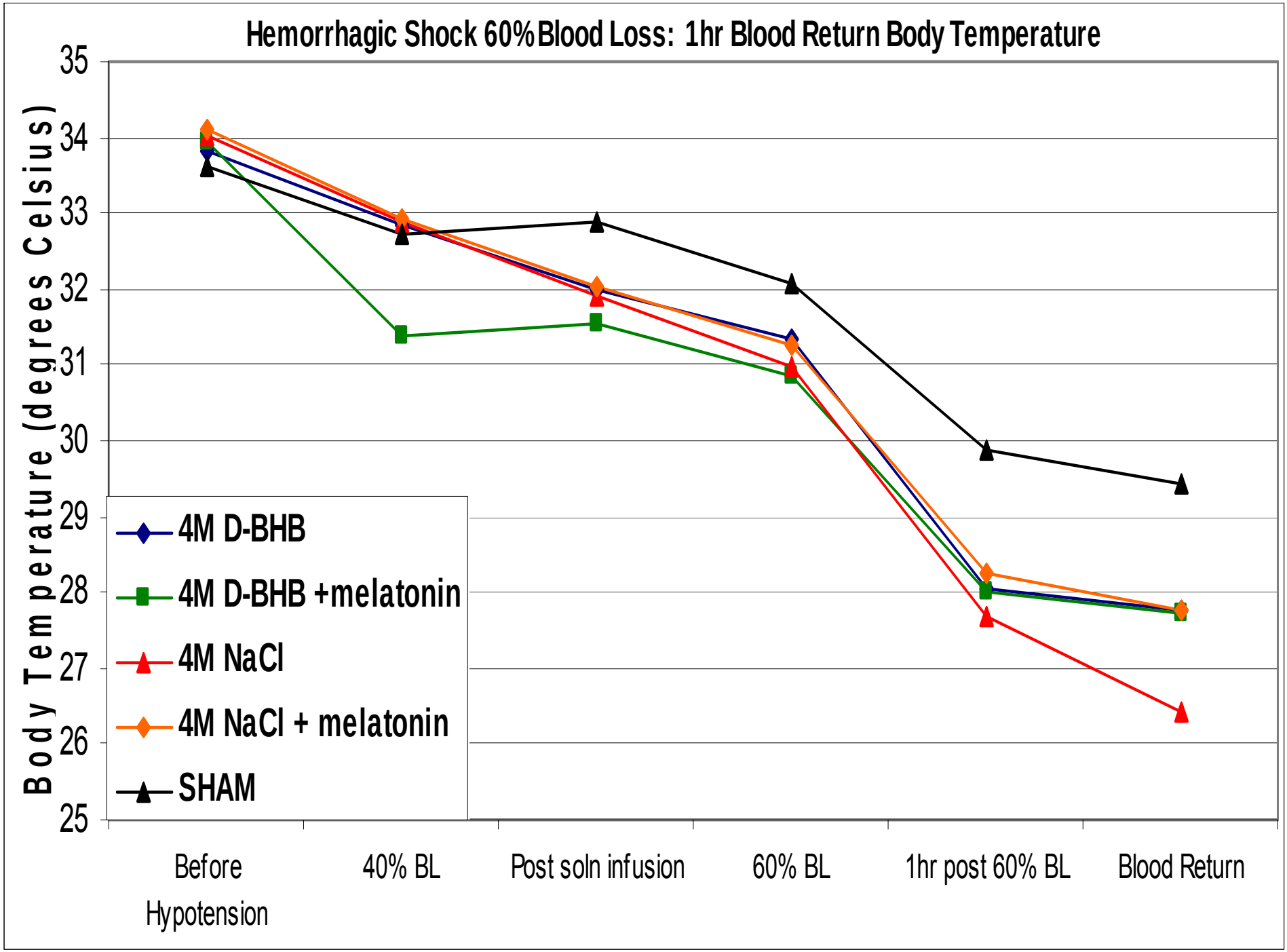

100
90
80
70
60
50
40
30
20
10
0
Percent Survival
SHAM
4M D-BHB +
melatonin
4M NaCl +
melatonin
4M D-BHB
4M NaCl
Blood Return
12 hours
24 hours
48 hours
72 hours
84 hours
10 days
Time Post Blood Return

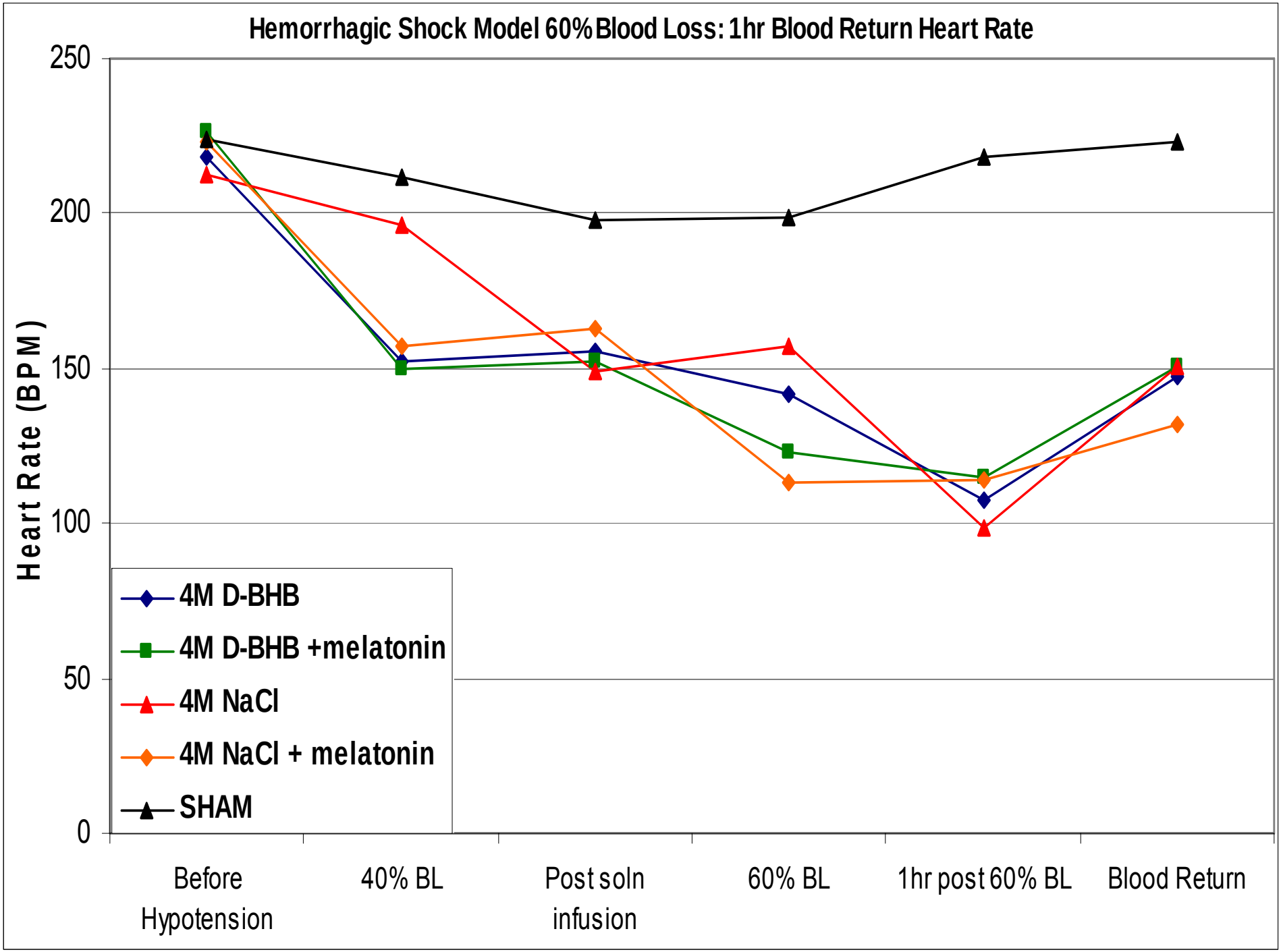

Serum BHB levels: Blood Return after 1 hr 60% Blood Loss
4M D-BHB
4M D-BHB + melatonin
4M NaCl
4M NaCl + melatonin
Sham
[BHB] (mmol/L)
0
1
2
3
4
5
6
7
8
Before Hypo
40% BL
Post Infusion
60% BL
60 Min Post 60%
Blood Return

- Does infusion of D-BHB improve physiological parameters upon 60% blood loss?
  - Mean Arterial Blood Pressure (MABP)
  - Body Temperature (T_b)
  - Heart Rate (BPM)

- Are these different from our control, NaCl?


Hemorrhagic Shock 60% Blood Loss: 1hr Blood Return Mean Arterial Blood Pressure
MABP (mmHg)
140
120
100
80
60
40
20
0
4M D-BHB
4M D-BHB +melatonin
4M NaCl
4M NaCl + melatonin
SHAM
Before Hypotension
40% BL
Post soln infusion
60% BL
1hr post 60% BL
Blood Return


Hemorrhagic Shock 60% Blood Loss: 1hr Blood Return Body Temperature
Body Temperature (degrees Celsius)
35
34
33
32
31
30
29
28
27
26
25
4M D-BHB
4M D-BHB +melatonin
4M NaCl
4M NaCl + melatonin
SHAM
Before Hypotension
40% BL
Post soln infusion
60% BL
1hr post 60% BL
Blood Return


Hemorrhagic Shock Model 60% Blood Loss: 1hr Blood Return Heart Rate
Heart Rate (BPM)
250
200
150
100
50
0
4M D-BHB
4M D-BHB +melatonin
4M NaCl
4M NaCl + melatonin
SHAM
Before Hypotension
40% BL
Post soln infusion
60% BL
1hr post 60% BL
Blood Return

Survival Experimental Conclusions

- Kaplan-Meirer graph shows 4M D-BHB + melatonin as greatest survival compared to SHAM animals (n=10)
- Addition of the antioxidant melatonin may play big role in aiding against reperfusion injury upon blood return
- Physiological parameters of MABP, T_b, and Heart Rate are generally the same with ketone and control infusions

Ongoing Experiments

- Survival Experiments: Data collection and analyze results
  - Neurological Scoring (Days 1-6)
    - Basic behaviors
  - Memory Testing (Days 7-10)
    - Higher order brain processing
  - Histology (Day 10)
    - Brain damage due to 60% blood loss for one hour